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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : C07D 493/22, A61K 31/335 | A1

(11) International Publication Number: **WO 00/63212**

(43) International Publication Date: 26 October 2000 (26.10.00)

(21) International Application Number: PCT/CN99/00123

(22) International Filing Date: 20 August 1999 (20.08.99)

(30) Priority Data:
99 1 05796.1 16 April 1999 (16.04.99) CN

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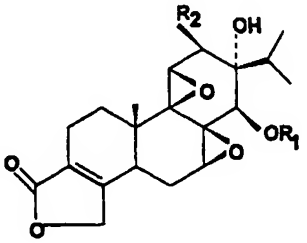
(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: DERIVATIVES OF TRIPTOLIDE, AND PREPARATION AND USES THEREOF



(1)

(57) Abstract

The present invention provides a compound having Formula (1) wherein R_1 is H, an alkyl containing 1–4 carbon atoms, –Ac, $-C(=O)(CH_2)_nCO_2$, or an amino acid, wherein n is an integer of 1–4; R_2 is –SCN, –NCS. The compounds significantly inhibit tumor cell growth both in vitro and in vivo.

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Derivatives of Triptolide, and Preparation and Uses Thereof

Technical Field of the Invention

The present invention relates to derivatives of triptolide, the method for producing the same and their uses in treatment of cancer diseases. The present invention also relates to the pharmaceutical compositions containing the derivatives of the invention.

Background of the Invention

Lei Gong Teng (Tripterygium wilfordii Hook F) is an herb with a variety of bioactivities, which can be used in traditional Chinese medicine. Several laboratories have demonstrated its anti-cancer activity. Kopachan et al. (Kopachan, SM, William AC, Richard GD et al., J. Am. Chem. Soc., 1972:94(2): 7194) isolated from Lei Gong Ten a diterpine containing epoxy (Triptolide) and demonstrated that 0.2 and 0.25 mg/kg of triptolide (intrapertoneal injection) can prolong the life time of L_{615} leukemia mice. 1ng/ml of triptolide can inhibit in vitro proliferation of KB cell of nasopharyngeal carcinoma (Zhang Qinmu et al, Zhong Guo Yao Li Xue Bao. 1981; 2:128). Triptolide inhibit colony formation of breast or stomach cancer cells to a similar extend as that of human leukemia HL-60 cells, and the IC_{50} is 0.504 -1.22μg/L. The experiment on effect of triptolide on cytokinetic of Hela cells indicates that triptolide has cytocidal effects on phase-synchronized cells, and cells at the S phase are most sensitive to triptolide. It has been demonstrated that triptolide inhibits synthesis of RNA and protein, selectively inactivates_sulfhydryl of phosphofructokinase, inhibits synthesis of liver glycogen, interferes with DNA replication (Xu Jianhua et al, Zhong Guo Yao Li Xue Bao. 1989; 10:550). Owing to epoxy diterpine isolated from Lei Gong Teng have multiple bioactivities, it has drawn great attention among scientists. The chemical synthesis, structure modification and bioactivities of it have been extensively investigated (Berchtold GA et al, J. Am. Chem. Soc. 1980; 102:1200). Synthesized triptolide was achieved in the early 1980s, but it involves numerous reaction steps and strict reaction conditions.

Yu Dequan et al (Yu Dequan et al, Yao Xue Xue Bao. 1992; 27:11) made structure modification of triptolide and obtained a number of valuable analogs. Because the effective bioactive dosage of triptolide is closed to its LD_{50}, it can not be applied to clinical practice. Due to unique immunomodulation mechanism of triptolide compared with other natural agents, currently most of researches are focused on its immunosuppressing effects, and few of them relate to its anti-cancer effects.

The advancement in the field of molecular oncology allows the use of oncogene as a novel target to which an anticancer drug directs. Bcl-2 is an oncogenic protein that inhibits programmed cell death. It involves the physiologic and pathological processes in organisms. Bcl-2 was also found to suppress the activation of caspases and became an important target for the therapy of tumors.

Apoptosis of tumor cells can be induced by triptolide (Tengchaicri T et al, Cancer Lett. 1998; 133:169). However, the mechanism involves the processes that have not yet been identified. The present invention provides a new anticancer agent that is an analog of the compound triptolide. The compound shows excellent cytocidal activity against cancer cells of renal, ovarian, melanoma, breast or colon origin, especially the cells overexpressing bcl-2 oncogene. The present invention also provides a method of producing the compound, and a new anti-tumor therapeutic strategy based on the use of the compound in combination with an antisense oligonucleotide that is an effective inhibitor of human cancer cell growth in vitro and in vivo.

It is an object of the present invention to provide a triptolide derivative with low toxicity and useful in clinical application.

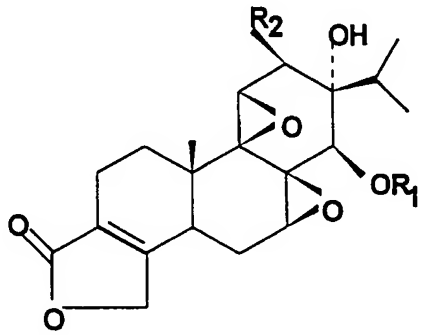
It is another object of the present invention to provide the uses of triptolide derivatives in the preparation of pharmaceutical compositions used in the treatment of cancers.

It is still another of the present invention to provide a method for producing the triptolide derivatives.

It is yet another object of the present invention to provide a pharmaceutical composition comprising the derivative of the present invention.

Detailed Description of the Invention

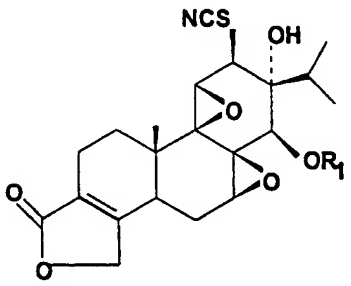
The present invention provides a triptolide derivative with the following formula:



wherein R_1 is H, alkyl containing 1-4 carbon atom(s), -Ac, -C (=O) $(CH_2)n$ CO_2, or an amino acid, wherein n is an integer of 1-4; R_2 is -SCN, or -NCS. Due to rearrangement of atoms within the molecule, -SCN and -NCS is interchangeable.

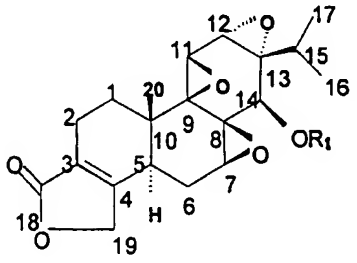
Preferably, R_2 is -SCN and R_1 is H.

Yu et al. opened the epoxide at C_{12} and C_{13} by using different agents and obtained a series of triptolide derivatives including tripchlororide and tripbromide. The halogen at C_{12} position may be removed in vivo under enzymatic catalysis, and the oxygen ring at C_{12} and C_{13} may be formed again and toxicity of triptolide may be recovered. The present invention uses ammonium thiocyanide to react with triptolide under mild condition to open epoxide at C_{12} and C_{13}, and obtains a compound, 12- -thiocyano-13- -hydroxy triptolide (hereinafter referred to as T12) with high anti-cancer activity and low toxicity. The compound thus obtained has the following formula:



wherein R_1 is H, alkyl containing 1-4 carbon atom(s), -Ac, -C (=O) $(CH_2)n$ CO_2, or an amino acid, wherein n is an integer of 1-4. R_1 can be added with known method.

The present invention also provides a method for producing the triptolide derivatives, comprising the step of reacting triptolide represented by the following formula with ammonium thiocyanide under heating conditions.



wherein R_1 is H, alkyl containing 1-4 carbon atom(s), -Ac, -C (=O) $(CH_2)n$ CO_2, or an amino acid, wherein n is an integer of 1-4.

Preferably, the reaction is performed in an organic solvent at a temperature of 65-87°C. More preferably, the organic solvent is t-butyl alcohol.

The present invention also provides the uses of the compound of the present invention in the preparation of pharmaceutical compositions for treatment of cancers.

In the above uses, the cancers to be treated may be cancers of breast, lung, renal, melanoma, colon or ovarian origin. Preferably, the cancers are of melanoma, renal, colon, ovarian and breast origin.

The present invention further provides a pharmaceutical composition comprising an effective amount of the derivatives of the invention for treatment of cancers, and pharmaceutical acceptable carriers or vehicles.

The cancers to be treated in the composition may be of breast, lung, renal, melanoma, colon or ovarian origin. Preferably, the cancers are of melanoma, renal, ovarian, colon and breast origin.

The present invention still provides a composition comprising the derivatives of the present invention and bcl-2 antisense oligonucleotides in a synergistic effective amount for treatment of cancers, and pharmaceutically acceptable carriers or vehicles.

Brief Description of the Drawings:

Figure 1 shows DNA degradation of T12-induced cell apoptosis;

Figure 2 shows T12-induced Bcl-2 cleavage in HL-60 cells;

Figure 3 shows comparison of Bcl-2 cleavage induced by T12 and other 3 compounds;

Figure 4 shows synergistic effect of T12 and bcl-2 antisense oligonucleotides on Col-06 cells.

The following experiments were made in accomplishing this invention.

Experiment 1: Preparation of 12- -thiocyano-13- -hydroxy triptolide

Triptolide (3.6g, 0.01 mole) was added to 400ml t-butyl alcohol under stirring at 50°C. Upon the triptolide was completely dissolved, 11.4g ammonium thiocyanide (0.15 mol) was added to t-butyl alcohol solution of triptolide in parts. Then the reaction mixture was stirred at 80°C for 24 hours. 400ml ethyl acetate was added after the reaction was completed. The organic phase was washed for three times with the saturated NaCl solution and dried with anhydrous $MgSO_4$. The filtered

solution was evaporated at 60°C on a rotary evaporator to remove the solvent and the residue was dissolved with acetone. The acetone solution was checked by TLC, developed in $CHCl_3/CH_3OH$ (95:5) solution. The 12- -thiocyano-13- -hydroxy triptolide was the major product on TLC with traces of triptolide and other by-products. 10g 70-140 μ silica gel was added to the acetone solution with stirring, and then the acetone was evaporated. Triptolide was first removed by silica gel (40-70 μ) with $CHCl_3$, and then eluted with $CHCl_3/CH_3OH$ solution (95:5). Fractions of T12 were collected under TLC-monitoring. Combine the T12 fractions, and the solvent was evaporated under vacuum. The residue was re-crystallized with acetone to obtain the crystal of 12- -thiocyano-13- -hydroxy triptolide (3.77g, 90% yield). mp: 265-266°C, Rf: 0.15 ($CHCl_3/CH_3OH$, 95:5), purple-red color in Kedd's reagent.

Elemental analysis: $C_{21}H_{25}NSO_6$, calculated value (%): C, 60.14. H, 6.01, N, 3.34, tested value (%): C, 59.85. H, 5.93. N, 3.26 $[\alpha]_D^{25}$ - 114.06°C (acetone, 4.17 mg/ml);

IR (KBr) cm^{-1} : 3450, 3000, 2152, 1739, 1674, 1441, 1032, 980. MS m/z (%): 419 (M^+, 13), 404 (-CH_3, 14.5), 361 ($-C(=CH_2)-CH_3$, 13), 343 (-H_2O, 13), 271 (14), 241 (23), 151 (48), 137 (89), 72 (91), 61 (100).

^{1}HNMR, ppm: 0.8 (3H, d, J=6.77Hz, 16-CH_3), 0.95 (3H, s, 20-CH_3), 0.99 (3H, d, J=6.88 Hz, 17-CH_3), 1.28 (1H, m, 1- H). 1.45 (1H, m, 1- H), 1.85 (1H, t, J=14.17 Hz, 6- H), 2.0 (1H, m, 2-H), 2.18 (1H, m, 2-H), 2.20 (1H, m, 6- H), 2.25 (1H, m, 15-H), 2.67 (1H, m, 5-H), 3.0 (1H, d, J=4.4Hz, 14-H), 3.35 (1H, m, 7-H), 3.75 (1H, d, J=5.61Hz, 11-H), 3.90 (1H, d, J=5.87Hz, 12-H), 4.85(2H, m, 19-H), 5.0 (1H, s, 3-OH), 5.45 (1H, d, J=4.2 Hz, 14-OH);

^{13}CNMR, ppm: 14 (16-C), 15.5 (20-C), 16 (17-C), 17 (2-C), 22.5 (6-C), 29 (15-C), 30 (1-C), 35 (10-C), 39 (5-C), 51 (11-C), 57.5 (12-C), 60.5 (8-C), 61.5 (7-C), 67 (9-C), 70.5 (19-C), 74 (14-C), 76.5 (13 -C), 115 (SCN), 123 (3-C), 162 (4-C), 173 (18-C).

Experiment 2: Inhibition of T12 on the growth of 6 human cancer cell lines in vitro

A detailed description of standard procedures and the method of data analyses performed by NCI have been published (Skehan P. et al., J. Nalt. Cancer Inst., 1990; 82:1107). The assay involves plating the cells, preincubating for 24 hours, followed by a 48 hours continuous drug exposure at appropriate concentration against 6 cell lines originated from 6 tumors (breast, colon, lung, melanoma, ovarian and renal). The cytotoxicity was assessed with the sulforhodamine B (SRB) protein assay. An evaluation parameter was used: The drug concentration which inhibits growth by 50% (GI_{50}). The growth inhibitory activity of the present invention (T12) was examined and compared with two other compound: Triptolide (T, leading compound of T12) and T8 (a derivative of T, see Jung, M. J., 1/1999 PCT/US98/08562). A known chemotherapeutic agent, taxol was also tested in the experiment, so as to compare the anticancer activity of known compounds and that of the compound in the present invention.

6 cell lines were grown in RPMI 1649 containing 5% fetal bovine serum and 5mM L-glutamine. T, T8 and T12 were tested for their effect on cell growth. Cells were harvested from exponential-phase maintenance culture, counted by trypan blue exclusion method, and dispensed on 96-well culture plates in 100 μl volumes. Following a 24 hr incubation at 37°C, 5% CO_2, 100% relative humidity, 100 μl of culture medium, the culture medium containing drug or the culture medium containing drug vehicle was dispensed within appropriated wells. Plates were then incubated for 48 hours. The growth inhibitory activity of T, T8, T12 were evaluated with SRB assay (Skehan P., 1990). Cell culture were fixed in site by adding 50 μl of cold 50% (wt/vol) trichloroacetic acid (TCA) and incubated for 60 minutes at 4°C. The supernatant was discarded and the plates were washed five times with deionized water and dried. 100 μl of SRB solution was added to each well, and the cultures were incubated for 10 minutes at room temperature. Unbound SRB was removed by washing four times with 1% acetic acid and the plates were air-dried. Bound dyes was solved with Tris buffer, and the optical densities were read on an automated spectrophotometric plate reader at a single wavelength of 490nm. GI_{50} were determined using NCI system. The results expressed in terms of GI_{50} are shown in Table 1.

Table 1. Effects of T12, T and Taxol on GI50 (μg/ml) of 6 cell lines

Tumor cell lines	T12	T	T8	Taxol
Bre-01 (breast)	0.055	0.04	0.3	0.06
Col-06 (colon)	0.03	0.02	0.1	0.007
Lu-06 (lung)	0.6	0.09	0.5	0.075
Mel-08 (melanoma)	0.09	0.02	0.25	0.07
Ov-01 (ovarian)	0.02	0.006	0.06	0.05
Re-01 (renal)	0.06	0.03	0.25	1

From Table 1, it can be seen that the compound of the present invention has a marked inhibition on all 6 cell lines, and the inhibition effects of T12 in renal cancer cells was significantly higher than that of taxol.

Example 3: Inhibitory effect of T12 on colony formation of renal cancer cell line

Clonogenic assays have the advantage of selecting tumor cells in a mixed population, since only the latter have the capacity to grow at low density. The assay has been widely used to assess the effects of tumor chemotherapy.

Re-01 cells were harvested from exponential-phase maintenance culture, counted by trypan blue exclusion, and dispensed in 6-well culture plates (50 cells/ well) in 0.5 ml volumes. Following a 24 hours incubation at 37°C, 5% CO_2, 100% relative humidity, 0.5 ml of culture medium, the culture medium containing drug or the culture medium containing drug vehicle was dispensed within appropriated plates. Culture plates were then incubated for 14 days. Cell colonies were fixed in site followed by Giemsa staining and counted under microscopy.

After using a colony forming assay with renal cancer cells and continuous drug exposure for 21 days, T12 showed significant inhibition effects (50% of growth inhibition) in renal cancer cell lines at 0.005 μg/ml of drug concentration.

Experiment 4: Inhibition of T12 on the growth of human melanoma in nude mice

L1210 and P388 leukemia were developed in 1945 and 1955 respectively, and played an important role in the screening of candidates of anticancer agents. However, whether P388 or L1210 is a poor predictor for solid tumor active drugs has yet to be investigated. A number of drugs, that are active against L1210 or P388, are inactive against experimental solid tumors. A nude mouse, deficient T-cells, cannot reject foreign tissues. If the nude mice are kept in a germ-free environment, the lifetime can nearly be increased to the length of a normal mouse, and the xenografts can produce many biological characters similar to those of human tumor tissue.

T12 was evaluated in nude mice against human melanoma xenografts. Female Bab/c nude mice, 6-8 weeks old, were obtained from Shanghai Institute of Pharmaceutical Industry, and randomly assigned to experimental groups of n = 6 nude mice. The tumor cells Mel-08 used were implanted subcutaneously into the auxiliary region ($1X10^7$ cells/0.2 ml/mouse). T12 treatment was initiated 24 hours after the implantation of the tumor cells. T12 (formulated in 0.5% of CMC-Na solution) were injected intraperitoneally for 14 days (4mg/kg body weight/day), and the tumor were removed at the indicated times. Dacarbazine (DTIC, 20 mg/kg/day) and 0.5% of CMC-Na solution (0.5 ml/mouse) were injected intraperitoneally as positive and negative control, respectively. Tumor inhibition (%) were calculated as follows:

$$[(C-T)/C]' 100$$

wherein C and T represent the mean tumor weight for control and treated groups, respectively.

DTIC and the compound of the present invention showed potent inhibitory effects (54.92% and 50.17%) on the growth of melanoma in nude mice, whereas no effects on tumor growth were observed for control group.

Experiment 5: Detection of fragmentation of genomic DNA by agrose gel electrophoresis

Apoptotic cell death involves the activation of a preprogrammed cascade of molecular events that culminate in DNA degradation, nuclear disintegration and packaging of the cell's remnants into membrane-enclosed "apoptosis bodies" which are subsequently removed by phagocytosis. Generally, agents who directly or indirectly cause DNA damage induce apoptosis.

The formation of distinct DNA fragments of oligonucleosomal size (180-200 bp lengths) is a biochemical hallmark of apoptosis in many cells. The DNA is cleaved between the nucleosomes resulting in a "ladder" of DNA fragments of multiples of 180-200 bp. The presence of extensive DNA breaks in apoptotic cells has provided the basis for the development of flow cytometric techniques to identify apoptotic cells and to determine their position in the cell cycle. The knowledge of the cell cycle phase specificity of antitumor drugs, therefore, is helpful in developing clinical protocols, especially involving drug combinations. Attempts have been made to classify the drugs according to whether they show selectivity toward noncycling or cycling cells or to particular phases of the cell cycle.

Following incubations with the designated concentrations and schedules of T12, 3 × 10^6 HL-60 cells were washed in PBS, and then lysed in 100 μl lysis solution (50mM Tris, pH 8.0; 10 mM EDTA and 0.25% NP-40) containing 3 μl proteinase K (1 mg/ml). The lysates were incubated at 37℃ for 30 minutes. Supernatants (20μl) were collected as described for agarose gel electrophoresis (Frank T, 1993).

FIG 1 shows that an exposure to 1 μg/ml T12 for 4, 12 and 24 hours (lanes 3-5) causes internucleosomal DNA fragmentation of the genomic DNA of HL-60 cells, and no internucleosomal DNA fragmentation of the genomic DNA for the control and 2 hours with T12 (lanes 1 and 2).

Experiment 6: Flow cytometry analysis of apoptosis and cell cycle

Apoptotic cells were detected by flow cytometry using P1 (Nicoletti, 1991). HL-60 lines, Re-01 cell lines with or without T12 were incubated at 37°C for 4-72 hours (2, 4, 8 and 24 hours for HL-60 cell lines; 12, 24, 48 and 72 hours for Re-01 cell lines). After centrifugation (700 g at room temperature for 5 minutes), the pellets were washed for two times and then resuspended in 50 μl of PBS. Aliquots containing 10^6 cells/ 50 μl were fixed in 70% ethanol at -20℃ overnight. Cells were washed and resuspended in PBS (0.5 ml) containing 0.25 mg/ml RNase and 0.1 mg/ml PI. Samples were kept in dark for 30 minutes. Cytometry was performed using the Epics Elite flow cytometer (COULTER, USA) equipped with a 488-nm argon-ion laser. Each of the analysis was performed with the Elite 4.0 and DNA Multicycle software. The percentages of G0/G1, S and G2/M cells were also calculated by cycle analysis on the gated apoptotic population.

Table 2 and 3 demonstrate respectively the induction of apoptosis in HL-60 and Re-01 cells following incubation with 1 μg/ml T12. The low molecular weight DNA from cells undergoing apoptosis following suspension in the phosphate-citrate buffer gave rise to a sub-G1 population on DNA content frequency histograms.

Table 4 and 5 show the distribution of HL-60 and Re-01 cells in various phases of the cell cycle after treatment with T12. Exponentially growing cells were treated with and without 1 μg/ml T12 for 4 hours. T12 increases the proportion of cells in the G0/G1 phases with 1 μg/ml for 2 hours and 4 hours, and decreases the population of cells in S/G2/M phases, indicating that apoptosis is selective to S phase cells.

Table 2. DNA content in apoptotic HL-60 cells following incubation with T12

Treatment time (h)	0	2	4	8	24
Sub-G1	9.1	28.9	74.8	79.8	79.8

Table 3. DNA content in apoptotic Re-01 cells following incubation with T12

Treatment time (h)	0	12	24	48	72
Sub-G1	5.1	10.3	27.4	54.0	90.0

Table 4. Cell cycle Distribution in HL-60 cells treated with T12

Treatment time	G0/G1	S	G2/M
Control	32.0	53.7	14.3
2	29.5	59.2	11.3
4	71.8	25.3	2.9

Table 5. Cell cycle Distribution in Re-01 cells treated with T12

Treatment time	G0/G1	S	G2/M
Control	28.4	60.6	11.0
24	38.1	36.9	25.0
48	40.7	43.3	16.0

Experiment 7: Western blot detection of Bcl-2 proteins expression

Apoptosis has become a basic tool in developing and establishing new strategies of cancer therapy. However, the molecular mechanism of T12-induced apoptosis is not well understood. The Bcl-2 protein plays an essential role in preventing cell death and is one of the major factors causing tumor formation. During the study of the molecular mechanisms of T12 action in Bcl-2-overexpressing cells, we found that T12 can induce the cleavage and inactivation of Bcl-2 in a caspase-dependent manner. Both biochemical and genetic evidence indicates that Bcl-2 can regulate cell death induced by caspases. Recent studies has shown that caspase-3 (CPP32) which can cleave poly(ADP-ribose) polymerase (PARP) and play an important role in triptolide-induced T lymphocyte apoptosis (Yang Yili et. al. Immunopharmacology 40: 139-149,1998).

Three cell lines (human leukemia HL-60 line, human breast cancer Bre-01 line and lymphoma Ly-01 line) were cultured at 1×10^6/ml and treated with 1μg/ml T12 for 24, 48 and 72 hours. Untreated cell lines were taken as control. Cells were washed and resuspended in lysis buffer containing 20 mM Tris.HCl (pH 8.0), 4% sodium dodecyl sulfate (SDS). Cell lysates were migrated on 12% SDS-polyacrylamide gel and transferred onto PVDF membrane. Membrane was blocked overnight with PBS containing 5% nonfat milk powder and incubated for 2 hours with anti-human Bcl-2 monoclonal antibody (Santa Cruz), followed by peroxidase-conjugated anti-Ig antibody. Detection was carried out with ECL solution.

As shown in Figure 2, treatment of HL-60 cells with T12 for 24 and 48 hours (lanes 4 and 5) lead to a cleavage of the 26kDa Bcl-2 into the 20 kDa product. Similar result was observed in Bre-01 cells (48 hours, lane 7). Under the same conditions, lymphoma cells did not produce a 20 kDa fragment (24 hours, lane 1), even when

cultured for 48 hours (lane 2). All treatment cell lines without T12 (lane 3 for lymphoma, lane 6 for HL-60, and 9 for Bre-01) were not changed for Bcl-2 expression.

To confirm Bcl-2 were also cleaved by other compounds (extract from Triptergium, triptolide and T8) to produce a 20 kDa fragment, we examined the Bcl-2 expression and compared the difference of Bcl-2 cleavage among the compounds in HL-60 cells. Figure 3 shows the cleavage of Bcl-2 during apoptosis by four compounds (lane 1-5: control, extract, T, T8 and T12). T12 (lane 5) displayed a more significant activity to induce Bcl-2 cleavage than other compounds.

Experiment 8: Synergistic effect of T12-combinated with bcl-2 antisense oligonucleotides

It was reported that bcl-2 antisense oligonucleotides are effective inhibitors of human tumor cell growth in vitro and in vivo. This antitumor activity was associated with an early down-regulation of bcl-2 expression (mRNA), followed by inhibition of cell growth and induction of apoptosis. It has been demonstrated that the combination of certain chemotherapeutic agents and bcl-2 antisense oligonucleotides shows a synergistic inhibitory effect on melanoma cells in vitro and in vivo (Campbell M J et al, British Journal of Cancer. 1998; 77:739-744). However, no studies have been reported on the efficacy of the combination of bcl-2 antisense and T12 that are directed to the same molecular target (protein of bcl-2).

Colon cancer Col-06 cells (10^6/mL) overexpressing bcl-2 cultured in RPMI-1640 medium supplemented with bovine serum, L-glutamine, and penicillin-streptomycin (100 μg/mL each) were treated with bcl-2 antisense (5μM). After 24 hours, T12 were added. The cytotoxicity of the antisense oligonucleotide or the antisense oligonucleotide + T12 in colon cancer cells was determined by SRB assay.

FIG 4 illustrates the combination of T12 and bcl-2 antisense oligonucleotide exerts significantly greater antitumor effects than either agent used alone on human colon and melanoma cell lines in vitro.

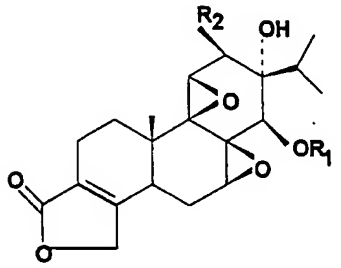
Experiment 9: Acute toxicity of T12

Kunming strain mouse (6 weeks old) was chosen for evaluating the acute toxicity of the compound of the present invention. Groups of mice (ten of each sex) were

injected intraperitoneally with T12 at doses of 40.96, 51.2, 64, 80, and 100mg/kg in 0.5% of CMC-Na solution. The mice were observed for two weeks for lethality. The LD_{50} (dose required to produce 50% lethality) were estimated to be approximately 74.42mg/kg using Bliss analysis. The LD_{50} of triptolide was reported to be 0.85mg/kg (Folkman J, Nature med 1995, 1:27). The result shows that the toxicity of T12 is significantly lower than that of T.

We claim:

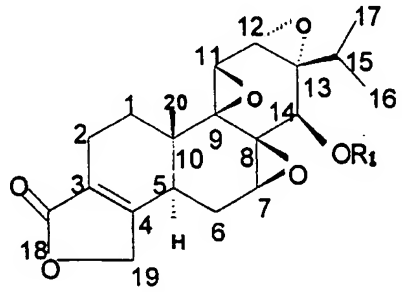
1. A compound which is a derivative of triptolide having the following formula:



wherein, R_1 is H, an alkyl containing 1 -4 carbon atoms, -Ac, $-C(=O)(CH_2)_nCO_2$, or an amino acid, n is an integer of 1-4; R_2 is -SCN, or -NCS.

2. The compound of claim 1, wherein R_2 is SCN, R_1 is H.

3. A method for preparation of the compound of claim 1, comprising the step of reacting the triptolide represented by the following formula with ammonium thiocyanide under heating condition,



wherein, R_1 is H, an alkyl containing 1 -4 carbon atoms, -Ac, $-C(=O)(CH_2)_nCO_2$, or an amino acid, n is an integer of 1-4.

4. The method of claim 3, wherein the reaction is carried out in an organic solvent at 65-87°C.

5. The method of claim 3, wherein the organic solvent is t-butyl alcohol.

6. Uses of the compound of claim 1 or 2 in the preparation of pharmaceutical composition for treatment of cancers.

7. The uses of claim 6, wherein said cancers are of ovarian, breast, lung, renal, melanoma, or colon origin.

8. A pharmaceutical composition, comprising the compound of claims 1 or 2 in an effective amount for treatment of cancers and a pharmaceutically acceptable carrier or vehicle.

9. The composition of claim 8, wherein said cancers are of breast, lung, renal, melanoma, colon or ovarian origin.

10. A pharmaceutical composition, comprising the compound of claim 1 or 2 and bcl-2 antisense oligonucleotide in a synergistic effect amount for treatment of cancers, and a pharmaceutically acceptable carrier or vehicle.

11. The composition of claim 10, wherein said cancers are of breast, lung, renal, melanoma, colon or ovarian origin.

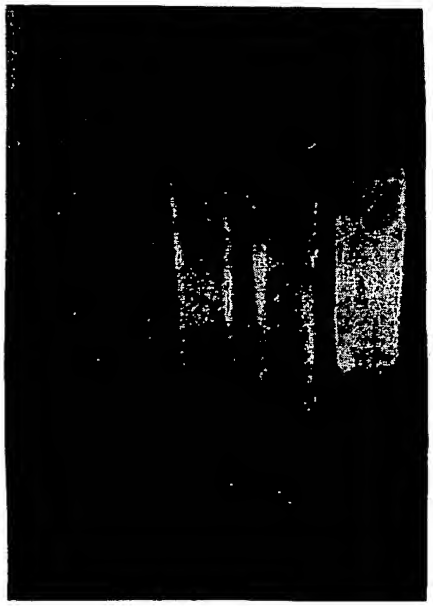


Fig.1

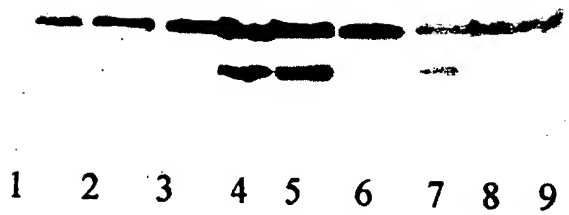


Fig.2

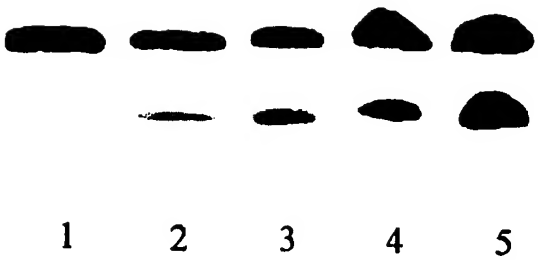


Fig.3

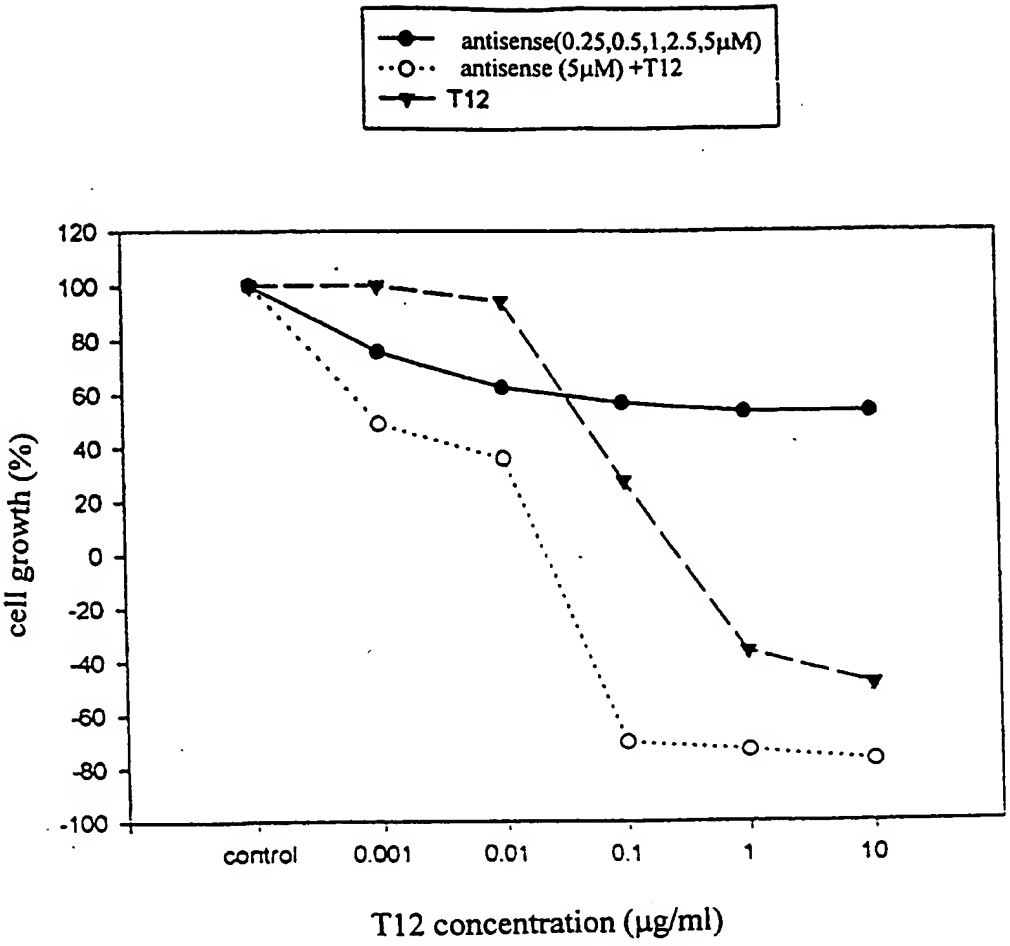

antisense(0.25,0.5,1,2.5,5μM)
antisense (5μM) +T12
T12
120
100
80
60
40
20
0
-20
-40
-60
-80
-100
cell growth (%)
control
0.001
0.01
0.1
1
10
T12 concentration (μg/ml)

Fig.4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN 99/00123

A. CLASSIFICATION OF SUBJECT MATTER

C07D 493/22;A61K31/335

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED C07D 493/22:A61K31/335

Minimum documentation searched (classification system followed by classification symbols)

PCT Minimum documentation(C07D 493/22;A61K31/335)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CNPAT(Triptolide);CAPLUS(constructional formula.Triptolide)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 97/31920	1-11
A	WO 97/31921	1-11
A	US 4,005,108	1-11
A	CN 1052859A	1-11
A	CN 1052860A	1-11
A	CN 1052861A	1-11

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim (S) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
06.Sep.1999(06.09.99)	23 SEP 1999 (23.09.99)
Name and mailing address of the ISA/CN 6 Xitucheng Rd., Jimen Bridge, Haidian District. 100088 Beijing, China Facsimile No. 86-10-62019451	Authorized officer Sen-chang Mu Telephone No. 62093870

Form PCT/ISA /210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN 99/00123

Patent document cited in report	Publication date	Patent family member(s)	Publication date
WO 97/31921	04.09.97	US 5663335	02.09.97
		WO 9731920	04.09.97
		AU 2061397	16.09.97
		AU 2125897	16.09.97